# A novel motif in the V3 domain of PKC-θ determines its immunological synapse localization and functions in T cells via association with CD28

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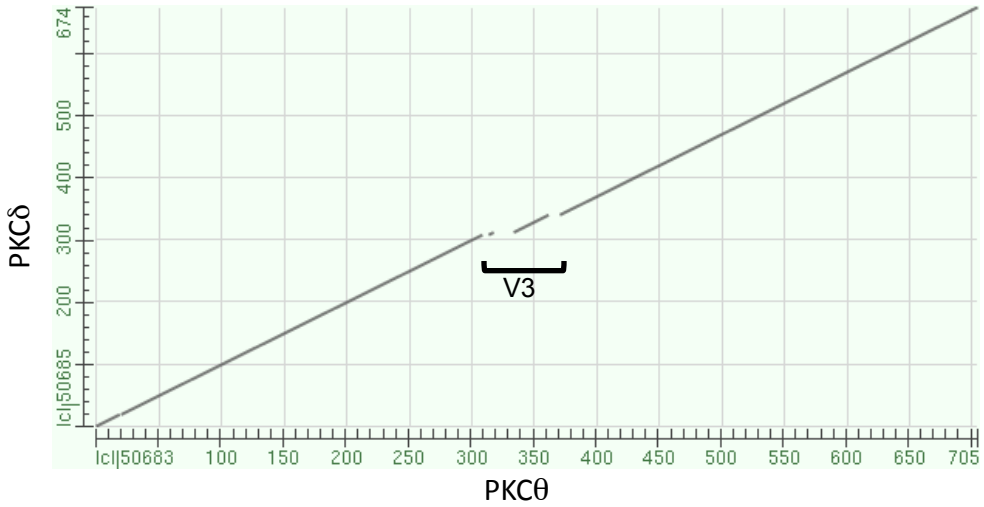
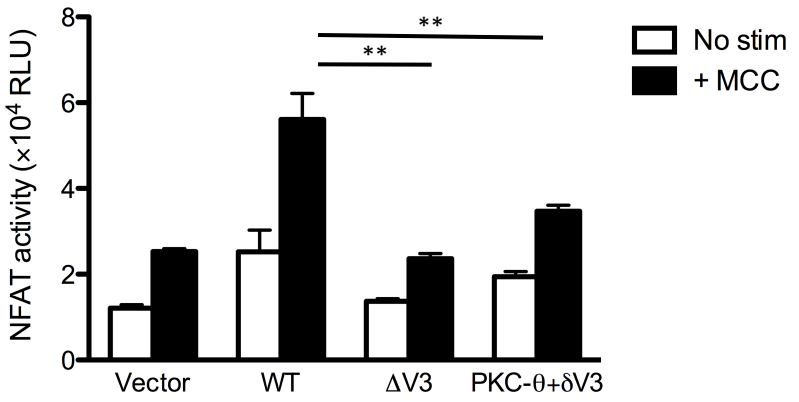
Supporting Online Materials
Supplementary Figures and Legends 1 – 9

**Figure S1**



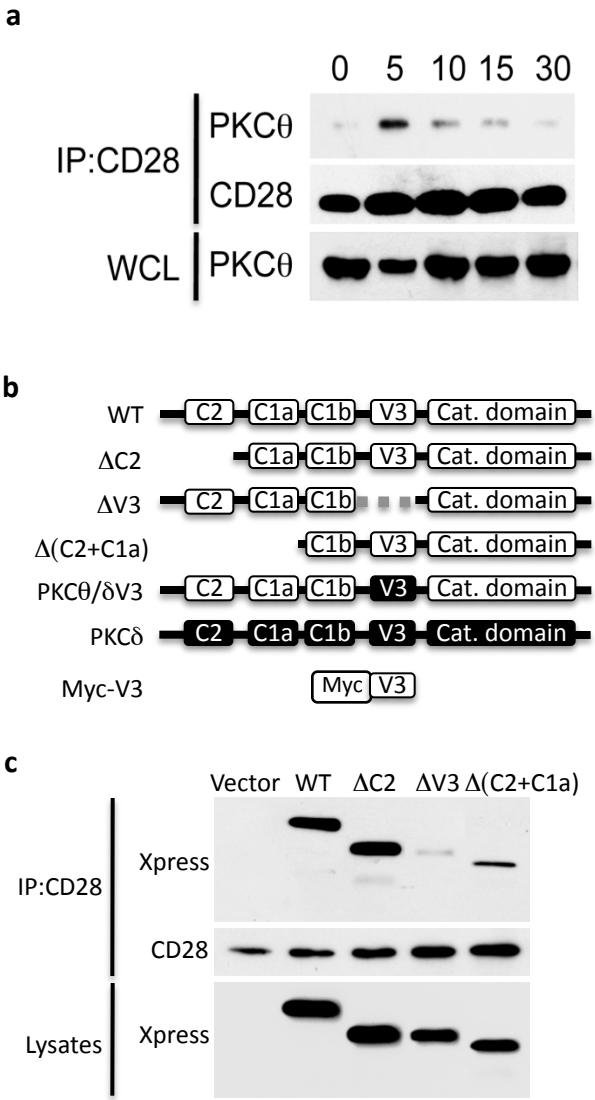

**Supplementary Figure 1**. Alignment of human PKC-θ (NP_006248) and PKC-δ (NP_006245) showing the divergence of their V3 (hinge) regions. Sequences were aligned using the NCBI BLAST program.

**Figure S2**




**Supplementary Figure 2**. Importance of the PKC-θ V3 domain for NFAT activation. Normalized Luc activity in MCC-specific T hybridoma cells cotransfected with empty pEF vector, WT PKC-θ, PKC-θ-ΔV3, or PKC-θ+δV3 together with NFAT-Luc and β-Gal reporter plasmids. Cells were cultured with DCEK fibroblasts expressing I-$E^k$ and B7-1 in the absence or presence of MCC peptide for 6 h. ** $p < .05$. Data are from two experiments.

**Figure S3**



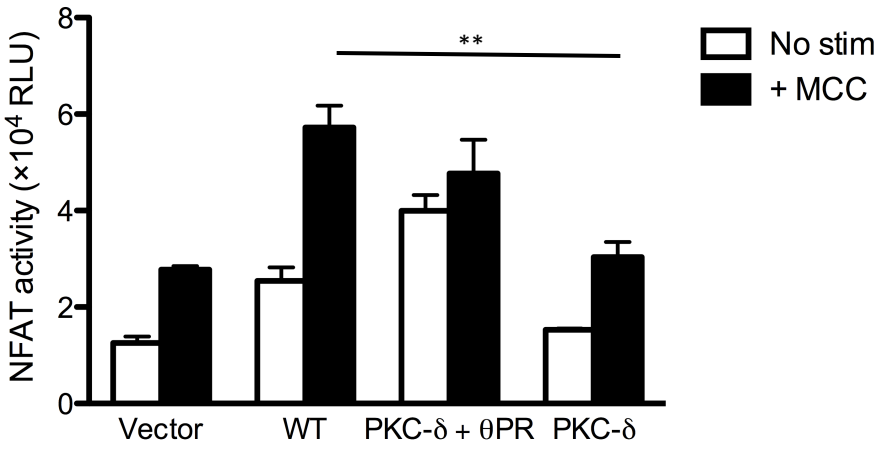

**Supplementary Figure 3**. The PKC-θ V3 domain interacts with CD28. (**a**) Immunoblot analysis of CD28 IPs or whole cell lysates (WCL) from Jurkat E6.1 T cells left unstimulated or stimulated for the indicated times (min) with anti-CD3 plus anti-CD28 mAbs. (**b**) Schematic representation of PKC-θ mutants. (**c**) Immunoblot analysis of CD28 IPs or lysates from Jurkat E6.1 T cells transfected with an empty vector or the indicated PKC-θ vectors, and left unstimulated or stimulated for 5 min with anti-CD3 plus anti-CD28 mAbs.

**Figure S4**


| Species | Sequence | Ensembl Accession |
|---|---|---|
| *Homo sapiens/Gorilla gorilla* | ARP**P**CL**P**TP | ENST00000263125 |
| *Pan troglodytes* | ARP**P**CL**P**TL | ENSPTRP00000041216 |
| *Macaca mulatta* | ARP**P**CL**P**TP | ENSMMUP00000027347 |
| *Canis familiaris* | ARL**P**CV**P**AP | ENSCAFP00000007725 |
| *Felis catus* | ARL**P**CV**P**AS | ENSFCAP00000008789 |
| *Equus caballus* | AKL**P**HA**P**AP | ENSECAP00000020818 |
| *Bos taurus* | AKP**P**YV**P**GP | ENSBTAT00000060978 |
| *Loxodonta africana* | TRL**P**YL**P**TP | ENSLAFP00000001356 |
| *Ailuropoda melanoleuca* | AKL**P**CV**P**AP | EFB18582.1 (NCBI) |
| *Mus musculus* | TRP**P**CV**P**TP | ENSMUST00000028118 |
| *Rattus norvegicus* | TRP**P**CV**P**TP | ENSRNOP00000025902 |
| *Ochotona princeps* | TRP**P**YL**P**TP | ENSOPRP00000002826 |
| *Dipodomys ordii* | TRQ**P**NF**P**TP | ENSDORP00000014980 |
| *Spermophilus tridecemlineatus* | ARP**P**YL**P**TP | ENSSTOP00000008114 |
| *Tupaia belangeri* | ARS**P**YL**P**TP | ENSTBEP00000011279 |
| *Procavia capensis* | TRL**P**YL**P**TP | ENSPCAP00000013723 |
| *Echinops telfairi* | TKL**P**YL**P**AP | ENSETEP00000013887 |
| *Cavia porcellus* | ARL**P**YL**P**TG | ENSCPOP00000013395 |
| *Dasypus novemcinctus* | TRL**P**YL**P**VP | ENSDNOP00000008621 |
| *Pteropus vampyrus* | ARP**P**HG**P**AL | ENSPVAP00000014575 |
| *Tursiops truncatus* | AKL**P**YG**P**AP | ENSTTRP00000012525 |
| *Xenopus laevis* | PKA**P**GL**P**MP | BAC79120.1 (NCBI) |
| *Danio rerio* | AIS**P**LT**P**AP | ENSDART00000046253 |
| *Tetraodon nigroviridis* | LLL**P**NL**P**LP | CAG04125.1 (NCBI) |
| *Takifugu rubripses* | VRA**P**SG**P**IT | ENSTRUP00000030203 |

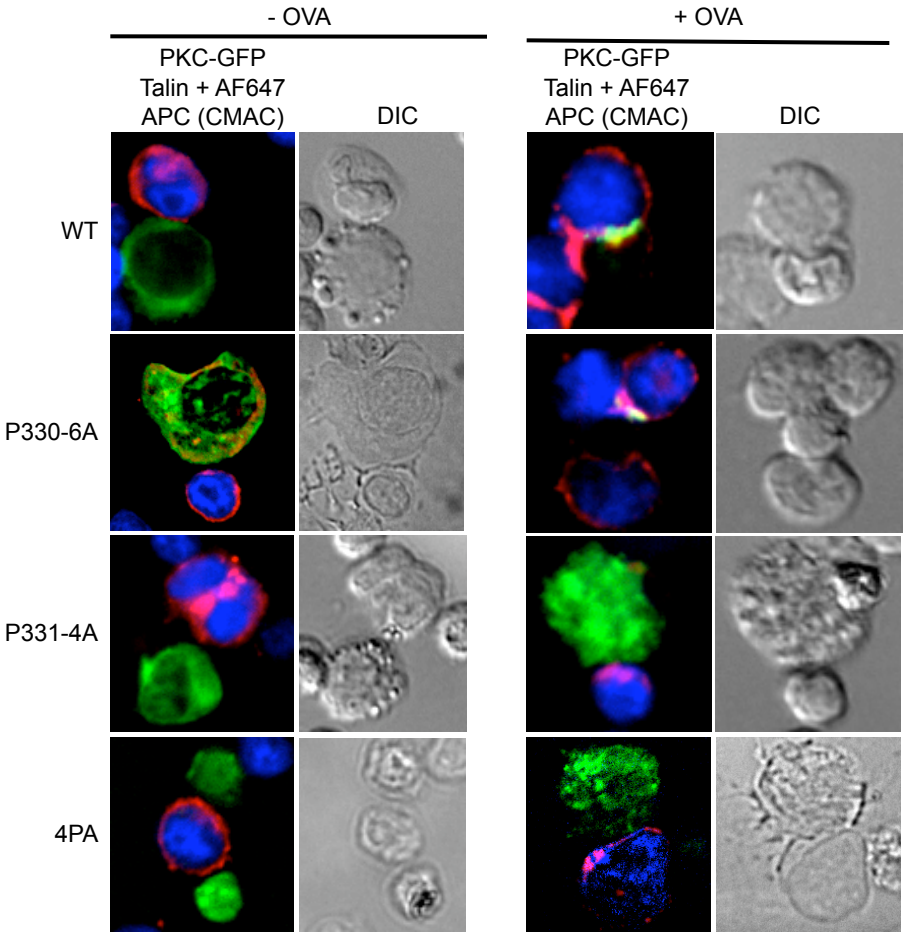
**Supplementary Figure 4**. Evolutionary conservation of the PxxP motif in the V3 domain of PKC-θ. Sequence alignment of the PR motif in putative PKC-θ enzymes from the indicated organisms. The human sequence corresponds to amino acid 328-336 of human PKC-θ. Alignment was performed using Weblogo (http://weblogo.berkeley.edu/logo.cgi). Proline residues that are absolutely conserved in all species are underlined in bold.

**Figure S5**



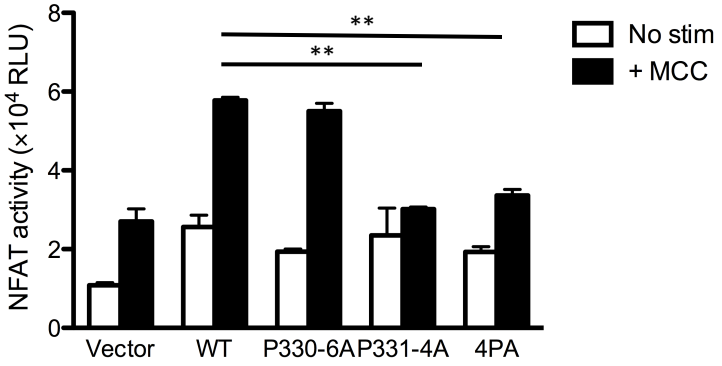

**Supplementary Figure 5**. Importance of the PR motif in the V3 domain of PKC-θ for NFAT activation. Normalized Luc activity in MCC-specific T hybridoma cells cotransfected with empty pEF vector or the indicated PKC-θ vectors together with NFAT-Luc and β-Gal reporter plasmids. Cells were cultured with DCEK fibroblasts expressing I-$E^k$ and B7-1 in the absence or presence of MCC peptide for 6 h. ** $p < .05$. Data are from two experiments.

**Figure S6**



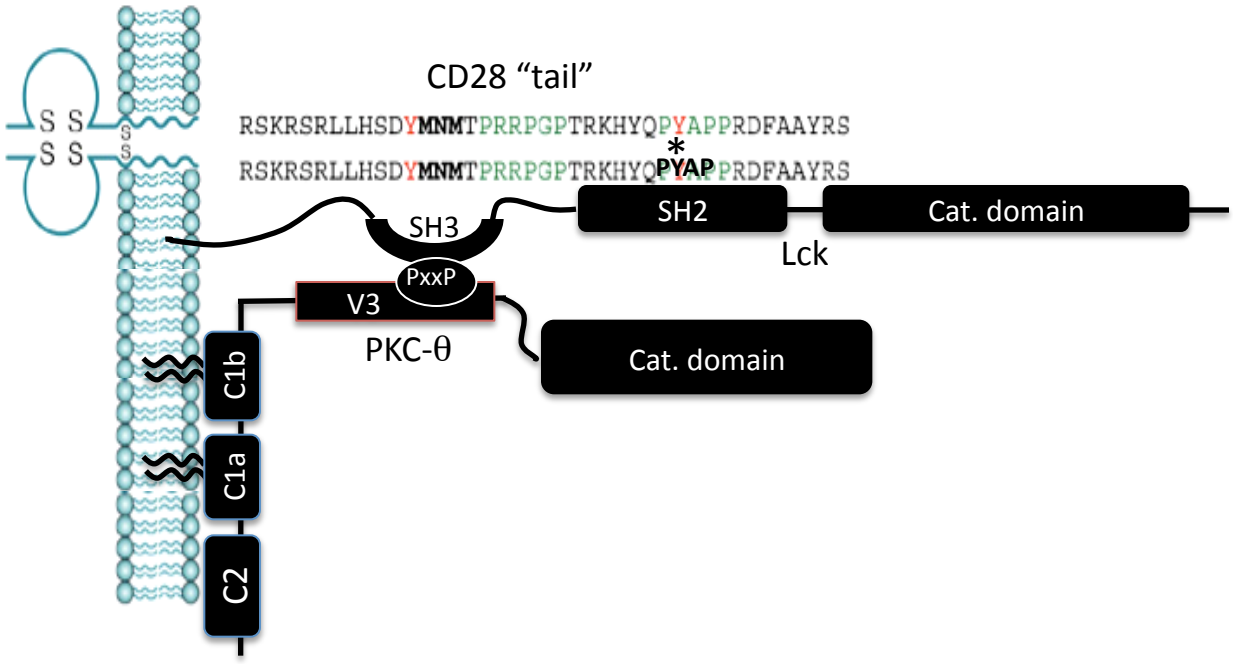

**Supplementary Fig. 6**. Importance of the PxxP motif in the V3 domain of PKC-θ for IS localization and CD28 interaction. *PKCθ*$^{-/-}$ OT-II CD4$^{+}$ T cells were infected with retrovirus expressing GFP-tagged PKC-θ, or PKC θ-GFP fusion vectors containing mutations at P330-6A, P331-4A, or all four proline residues (4PA) (green). Cells were fixed, stained and analyzed as in **Fig. 1a**.

**Figure S7**



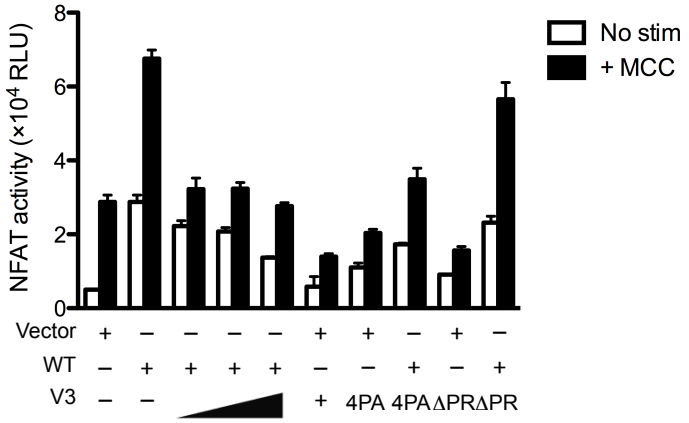

**Supplementary Figure 7**. A PxxP motif in V3 affects PKC-θ-mediated NFAT signaling. Normalized Luc activity in MCC-specific T hybridoma cells cotransfected with empty pEF vector or the indicated PKC-θ vectors together with NFAT-Luc and β-Gal reporter plasmids. Cells were cultured with DCEK fibroblasts expressing I-$E^k$ and B7-1 in the absence or presence of MCC peptide for 6 h. ** $p < .05$. Data are from two experiments.

**Figure S8**




**Supplementary Figure 8**. Schematic mode of tri-partite interaction between CD28, Lck and PKC-θ. Lck mediates the interaction between CD28 and PKC-θ, with its SH3 domain binding the PR motif in the V3 domain of PKC-θ, and its SH2 domain binding phosphorylated Tyr-207 in the CD28 $P^{206}Y^{*}AP^{209}$ motif.

**Figure S9**




**Supplementary Figure 9**. The PKC-θ V3 domain interferes with PKC-θ-mediated NFAT activation. Normalized Luc activity in MCC-specific T hybridoma cells cotransfected with empty pEF vector or the indicated combinations of full-length PKCθ and/or PKCθ-V3 vectors together with NFAT-Luc and β-Gal reporter plasmids. Cells were cultured with DCEK fibroblasts expressing I-$E^k$ and B7-1 in the absence or presence of MCC peptide for 6 h. Data are from two experiments.